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Investigation of the in vitro antioxidant activity of fermented tomato pruning residues and its effects on biochemical and hematological parameters of rats

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Abstract: In the tomato greenhouse production process, a by-product emerges in the form of greenhouse residues. One of these residues is leaf pruning residue, which poses an environmental pollution problem in terms of waste management. The purpose of this work was to investigate the use of fermented tomato leaves (FTL) as an animal feed additive and its potential to mitigate the environmental pollution problem caused by greenhouse pruning residues. The pruning residues were fermented and their in vitro antioxidant activity (AOA) levels were analyzed using 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH), reducing power (RP), and total phenolic content (TPC) assays. Wistar albino rats were fed diets containing standard rat chow with 25% FTL pellet. Performance parameters (feed intake, water intake, and body weight gain) of the rats were recorded for 21 days. Plasma biochemistry, hematological profile, and blood redox parameters (total oxidant status, TOS; total antioxidant status, TAS) were measured in rats euthanized after 21 days of treatment. It was determined that FTL had high AOA, which was due to nonphenolic components in FTL. There was no adverse effect of the test diet on the biochemical and hematological profile, and redox parameters, while an increase was observed in the feed intake. This study shows that FTL has nonphenolic AOA in vitro and that the diet prepared with 25% FTL has no detrimental effect on the measured parameters and can be used as an animal feed additive.

Key words: Body weight gain, environmental pollution, feed intake, greenhouse residues, in vitro antioxidant activity, leaf pruning residue

1. Introduction

Tomato plants are agricultural products containing abundant antioxidants and phenolic compounds. They are also rich in bioactive components, such as gallic acid, chlorogenic acid, ferulic acid, caffeic acid, rutin, and quercetin [1]. The phenolic contents of tomato plants are related to their antioxidant capacity and have protective effects from cellular oxidative stress when consumed [2]. These compounds can be found in the tomato fruit, as well as the root, leaves, and stem parts [1]. It has been reported that there is a relationship between the antioxidant capacity of tomatoes and the prevention of cardiovascular diseases and the risk of developing cancer [3]. The food industry mostly utilizes the fruit part of the plants and discards the rest as a by-product [4].

Bioactive components in tomato leaves can be obtained through various extraction methods. It is claimed that these components can be used as food additives as well as for pharmaceutical purposes [5].

Tomato pruning residue is often used to feed livestock with no economic benefit [1]. In a study conducted on rats, it is suggested that tomatine forms an unabsorbable complex with cholesterol in the gastrointestinal tract, interfering with cholesterol absorption and increasing sterol excretion [6]. In a study on hamsters, it was reported that the tomatine component added to the diet causes lower oral toxicity even at increasing doses compared to other glycoalkaloids and that most of the tomatine is excreted in the form of the tomatine-cholesterol complex without being absorbed from the intestines, having a lowering effect on LDL cholesterol [7].

This study aimed to investigate the in vitro antioxidant activity (AOA) of pruning residues and their use as a feed additive in animals, based on the idea that the recycling of tomato greenhouse waste will create both environmental and economic added value. To this end, we discussed the in vitro antioxidant effects of fermented tomato leaves

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(FTL) and the effects of FTL feeding on some biochemical, hematological, redox, and performance parameters (feed intake, water intake, and body weight gain).

2. Materials and methods

2.1. Processing of tomato leaves

Processing of tomato (*Solanum lycopersicum*) leaves for fermentation was carried out by a local company (Kozanlar Group, Sandıklı, Afyonkarahisar, Turkey) during the vegetation season of the plant. The processes involved sorting, crushing, dewatering, fermentation, drying, and pelleting. For the chemical composition of fermented tomato leaf pellets, a previous study by Aydoğan (2022) can be consulted (Table 1). It was reported that the FTL was rich in dry matter level, crude protein level, and some amino acids compared to the prefermentation process [8]. Fermented tomato leaf pellets were prepared as isocaloric and isonitrogenous to meet the nutrient requirements of the animals [9] (Table 2). An isocaloric diet provides a consistent number of calories per day, while an isonitrogenous diet provides a consistent amount of protein per day, regardless of other dietary factors. Both types of diets are commonly used in research studies to investigate the effects of various dietary factors on health outcomes.

2.2. Preparing FTL extracts for in vitro analysis

Fermented tomato leaf pellets were crushed into a powder and dissolved in ultrapure distilled water to a concentration of 45 mg/mL (w/v). This suspension was shaken well and poured into a Teflon microwave vessel. Extraction was performed in a microwave oven (Sineo MDS-10, Shanghai, China) according to an earlier method [10] (Temperature: 180 °C, Time: 20 min, Power: 400 W). The particulate extract solution was filtered with 0.45 µm polytetrafluoroethylene filter. The filtrate was stored at 4 °C for later use as a stock solution.

Fresh working solutions were prepared from the stock solution for in vitro analysis. For this, 1 mL of the stock solution was transferred to an Eppendorf tube and centrifuged (Ortoalresa Digicen 21R, Madrid, Spain) at 5000 *g* for 10 min. Using ultrapure distilled water, six sample series (1.41–45 mg/mL) were formed from this filtrate at a 1:2 dilution.

All solutions used for analysis were prepared and used fresh on the day of analysis, and determinations were made in triplicate. Online tools (Quest Graph™ IC_{50} Calculator, CA, USA; Gain Data ELISA Calculator, Hsinchu City, Taiwan) were used to calculate EC_{50} values.

2.3. ABTS radical scavenging activity assay

Thanks to the radical scavenging activity of ABTS radical cation ($ABTS^{\bullet+}$) in extracts, a reduced ABTS product is formed and chromophore change can be detected spectrophotometrically [11]. For this purpose, 150 µL of the ultrapure distilled aqueous dilutions of standard Trolox (7.81–250 mg/mL) and 6 sample series (1.41–45 mg/mL) were taken and the same volume of acetate buffer (mol/l, pH: 5.8) and 45 µL $ABTS^{\bullet+}$ (30 mmol/L) were mixed with

Table 1. Chemical composition of the fermented tomato leaves pellet.

Content	Amount
Dry matter, %	92.58
Crude protein, %	23.96
Nonprotein nitrogen, %	4.40
Crude ash, %	24.40
Crude oil, %	2.08
Crude fiber, %	15.54
Nitrogen free extract matter, %	25.71
Beta carotene, µg/100 g	8304
L-ascorbic acid, mg/kg	-
Alpha-tocopherol, mg/kg	50.88
Acid-detergent fiber, %	35.31
Neutral detergent fiber, %	44.57
Acid detergent lignin, %	12.09
In vitro neutral detergent fibre digestibility (48h), %	26.47
Pellet durability index, %	98.91

The chemical compositions and content quantities of the fermented tomato leaf pellet are presented as indicated.

Table 2. Content and chemical composition of the fermented tomato leaves diet.

Diet	**C**	**FTL**
Razmol, %	1.0	1.0
Barley, %	17.9	6.0
Corn, %	21.7	21.7
Vegetable oil, %	4.4	4.4
Sunflower seed meal, %	18.0	11.4
Soy sauce, %	32.0	6.0
DCP, %	2.5	2.5
Limestone, %	1.0	0.5
Salt, %	1.2	1.2
Vitamin mineral mix, %	0.3	0.3
Fermented tomato leaf, %	-	25.0

Dietary compositions are shown as percentages. Abbreviations: C, Control group (standard rat chow); FTL, Fermented tomato leaves fed group; DCP, Dicalcium phosphate.

96 test plates ($ABTS^{\bullet+}$, Trolox, and buffer are reagents of the total antioxidant capacity test kit, Rel Assay Diagnostics LLC, Gaziantep, Turkey). 150 µL acetate buffer and 45 µl $ABTS^{\bullet+}$ were used to obtain the control absorbance (A_C) value. Then, the plate surface was covered with a plastic seal and incubated at 30 °C for 10 min and the absorbances of the standards (A_S) and samples were read at 734 nm (Fisher Scientific-AccuSkan-FC). The percent inhibition of $ABTS^{\bullet+}$ was calculated with the formula below, and EC_{50} values were calculated as mg/mL.

$$\%\text{ABTS inhibition} = [(A_C - A_S) \times (A_C)^{-1}] \times 100$$

2.4. DPPH radical scavenging activity assay

According to a previous study [12], 30 µL of the extract (1.41–45 mg/mL) and standard Trolox (7.81–250 mg/mL) dilution series were mixed with 270 µL of the ethanolic DPPH (6×10^{-5} M) solution in a 96 test plate. After 30 min of incubation, the absorbance of the control (A_C, DPPH solution) and the absorbance of the standard and samples (A_S) were read at 520 nm. Determination was obtained at constant room temperature and in the dark. The percent inhibition of DPPH was calculated with the following formula, and EC_{50} values were calculated as mg/mL.

$$\%\text{DPPH inhibition} = [(A_C - A_S) \times (A_C)^{-1}] \times 100$$

2.5. Reducing power assay

According to the previously suggested methods [13], 25 µL of extract dilution series (1.41–45 mg/mL) were taken into Eppendorf tubes. It was mixed with the same volume of sodium phosphate buffer (200 mM, pH 6.6) and 1% (w/v) potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. Then, 25 µL of 10% (w/v) trichloroacetic acid was added and centrifuged (5000 *g*, 10 min). Fifty µL of the supernatant was taken and mixed with the same volume of distilled water, and 0.1% (w/v) ferric chloride was added and incubated at room temperature for 10 min. The absorbance was read at 700 nm. The percent reducing the power of the sample (A_S) was compared with the maximum absorbance (A_M) of Trolox dilutions (7.81–250 mg/mL) and was calculated using the formula below.

$$\%\text{Reducing power} = [(A_S) \times (A_M)^{-1}] \times 100$$

A_M: max Trolox absorbance; A_S: absorbance of the sample.

2.6. Total phenolic content assay

A modified combination of the Office International de la Vigne et du Vin and Rosbash (1949) methods was used to quantify the total amount of phenolic compounds [14].

2.6.1. Preparing the Folin-Ciocalteu reagent

One hundred g of sodium tungstate and 25 g of sodium molybdate were dissolved in 700 mL of distilled water. Next, 50 mL of 85% phosphoric acid (v/v) and 100 mL of concentrated (38%) hydrochloric acid were added and titrated with 30% (v/v) hydrogen peroxide in a beaker until the yellow color characteristic of Folin-Ciocalteu Reagent (FCR) was obtained.

Ten µL of the extract dilution series (1.41–45 mg/mL) were taken into Eppendorf tubes and mixed with 500 µL of distilled water, 50 µL of FCR, and 200 µL of 20% sodium carbonate (w/v). Thirty min of incubation at room temperature was provided for the stabilization of the reaction. The absorbance was read at 750 nm. The percentage of total phenolic substance content of the sample was compared with the maximum absorbance of Trolox dilutions (7.81–250 mg/mL) and calculated using the following formula.

%Total phenolic content = $[(A_S) \times (A_M)^{-1}] \times 100$

A_M: max Trolox absorbance; A_S: absorbance of the sample.

2.7. In vivo experimental stage

The study utilized a total of 14 Wistar albino male rats, with an age range of 3–4 months and a body weight (BW) of 360 ± 30 g. The difference in the initial weights of the rats was taken into account as a covariate in the statistical analysis to eliminate the effects of weight differences on the parameter changes. The experimental protocol was approved by the Afyon Kocatepe University Animal Ethics Committee (ref. no: AKUHADYEK-205/20). All rats were housed in standard rat cages one week before the study for adaptation. On the first day of the study, the rats were divided into two groups (n = 7) as the Control (C) and Test group (T), and were placed in separate cages. The C group rats were fed with standard rat chow and the T group rats were fed with FTL pellets. Fermented tomato leaf pellets were prepared fresh daily. Feed and tap water were kept in the cages ad-libitum. The animals' normal standard rat feed consumption habits were taken into consideration in determining the route and dose of administration. For this purpose, the ad-libitum feeding method was preferred. The total study period lasted 21 days, during which the rats were kept in stable conditions (12 h dark/light cycle; 20 ± 1 °C, 65% humidity).

During the study period, the animals' feed and water consumption parameters were recorded at 9:00 a.m. every day, and weight measurements were made. At the end of the study, the animals were sacrificed using appropriate methods (intramuscular injection of 10 mg/kg Xylazine HCl and 50 mg/kg Ketamine HCl anesthesia), and the samples were sent to the laboratory. The sampling procedure was as follows: An average of 6–9 mL of blood was taken by exsanguination from the hearts of the rats, labeled, and quickly transferred to heparinized tubes and sent to the laboratory. In the laboratory, hematological parameters in whole blood, biochemical and total oxidant status (TOS), and total antioxidant status (TAS) parameters in plasma were measured.

2.8. Analyses of biochemical and hematological parameters

For the biochemical profile, the parameters of glucose, total bilirubin, creatinine, urea nitrogen, uric acid, total protein, total cholesterol, triglyceride, high-density lipoprotein (HDL-cholesterol), low-density lipoprotein (LDL-cholesterol) levels, alanine transaminase (ALT), and aspartate transaminase (AST) enzyme activities were measured on an auto-analyzer (Cobas Integra 400 Plus, Roche Ltd, Switzerland) using commercial Roche kits. Measurement of hematological parameters (WBC, white blood cells; LYM, lymphocytes; MID, mid-range absolute count; GRA, granulocytes; Hb, hemoglobin; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RBC, red blood cells; MCV, mean corpuscular volume; RDWs, red cell distribution width standard deviation; RDWc, red cell distribution width variation coefficient; HCT, hematocrit; PLT, platelets; PCT, plateletcrit; PDWs, platelet distribution width standard deviation; PDWc, platelet distribution width variation coefficient; MPV, mean platelet volume) was performed on a hematology analyzer (Mindray BC-2800 Vet, Shenzhen Mindray Animal Medical Technology Co., China) using commercial kits.

2.9. Measurement of blood redox parameters

The redox status of the organism was determined by measuring TOS and TAS colorimetric assay kits (Rel Assay Diagnostics LLC, Gaziantep, Turkey) from blood samples. These kits were used to quantitatively determine the redox substances in the blood.

2.10. Performance parameters

Performance parameters, including feed intake (FI), BW, and body weight gain (BWG), were measured.

2.11. Statistical analysis

The results of all treatments for in vitro analyses were presented as mean ± standard deviation. Analyses were performed using ANOVA and post-hoc Tukey HSD in SPSS (IBM ver. 20, NC, US) software. The relationship between antioxidant parameters was examined using the Pearson correlation method. In evaluating the in vivo data, mixed linear modeling was used with the PROC MIXED command in the SAS program (SAS Institute Inc., NC, US). In repeated measurements for feed consumption, water consumption, and body weight values, fixed effects were determined as application, sampling time, and application × time interaction. In repeated measurements, the random effect for water consumption and feed consumption was determined as the cage; the random effect for live weight was determined as the rat. By trying different covariate values in time-dependent values, the covariate model with the smallest Bayesian information criterion for each parameter was taken into account. In the evaluation of blood parameters, mixed linear modeling was used with the PROC MIXED command. In the model, the fixed effect was determined as the application and the random effect. In all models, degrees of freedom were calculated according to the Between-Within approach. During the analysis of the data, standardization of residual values (Studentized Residual) was applied, and samples with residual values less than –4 or greater than 4 were considered marginal values (outliers) and were excluded from the model. The initial body weights of the rats were included in the model as covariate values. Values in all tables and graphs were expressed as least-squares means (LSMEANS) and SEM. The significance level was determined as $p < 0.05$.

3. Results

The AOA values of the in vitro radical scavenging activities of antioxidants were determined by ABTS, DPPH, and RP assays, and their phenolic contents (mg TE/mL extract) were determined by the TPC assay and presented in Figure 1.

ABTS radical scavenging activity levels of FTL dilutions varied between 83.90% and 66.22%. FTL showed the highest AOA at 5.63 mg/mL and the lowest at 1.41 mg/mL concentration ($p < 0.001$). The curve of the dilutions showed a cubic trend ($R^2 = 0.961$). It has been determined that the EC50 for the ABTS assay is higher than the standard (27.0 ± 1.3; 14.8 ± 1.8, respectively).

Values in the DPPH assay varied between 88.73% and 43.84%. FTL showed the highest AOA at 45 mg/mL and the lowest at 1.41 mg/mL concentration ($p < 0.001$). The curve of the dilutions showed a cubic trend ($R^2 = 0.996$). It has been determined that the EC_{50} for the DPPH assay is lower than the standard (5.1 ± 0.8; 68.0 ± 12.7, respectively).

In the RP assay, the values varied between 95.58% and 27.44%. FTL showed the highest AOA at 22.5 mg/mL and the lowest at 1.41 mg/mL concentration ($p < 0.001$). The curve of the dilutions showed a cubic trend ($R^2 = 0.998$). It has been determined that the EC_{50} for the RP assay is lower than the standard (3.32 ± 0.40; 158.9 ± 0.5, respectively).

The values in the TPC assay varied between 28.81 and 21.17 mg TE/mL extract ($p < 0.001$). The highest phenolic content was determined at 45 mg/mL and the lowest at 5.63 mg/mL concentration. The curve of the dilutions showed a quadratic trend ($R^2 = 0.971$). It has been determined that the EC_{50} for the TPC assay is lower than the standard (12.22 ± 5.0; 144.31 ± 6.4, respectively).

The results of the Pearson correlation analysis, which was performed to better analyze the extent to which the assays reflect the AOA and the relationship between TPC and the assays, are presented in Table 3. According to these results, a small-moderate relationship was found between TPC and ABTS, between TPC and DPPH, and between TPC and RP, but it was not statistically significant ($r(18) = 0.16$, $p = 0.519$; $r(18) = 0.46$, $p = 0.225$; $r(18) = 0.40$, $p = 0.229$, respectively). There was a highly significant positive correlation ($p < 0.01$) between ABTS and DPPH, ABTS and RP, and DPPH and RP ($r(18) = 0.64$; $r(18) = 0.79$; $r(18) = 0.90$, respectively).

Biochemical analysis results are presented in Table 4, hematological analysis results in Table 5, analysis results of redox parameters in Table 6, feed consumption in Figure 2, water consumption in Figure 3, and weight gain parameters in Figure 4.

As presented in Table 4, we compared the biochemistry parameters of the control group rats and found that FTL did not affect enzyme activities (ALT and AST) and routine biochemical parameters (glucose, HDL-cholesterol, LDL-

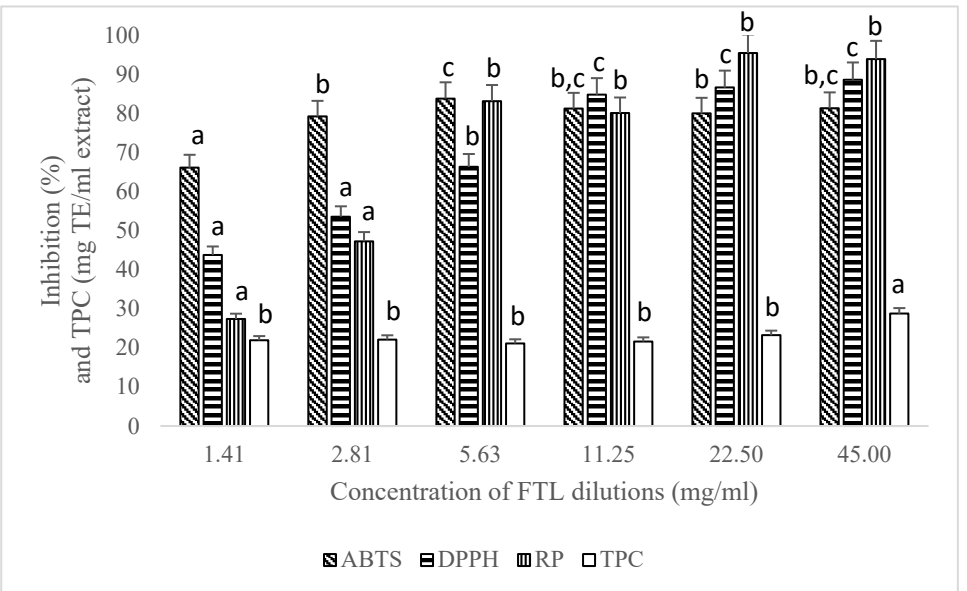

Figure 1. Analysis of antioxidant activity levels and total phenolic content of fermented tomato leaves. Antioxidant activity (ABTS, ABTS radical scavenging assay; DPPH, DPPH radical scavenging assay; RP, reducing power assay) levels and total phenolic content (TPC) analysis of FTL at different concentrations (data expressed as mean ± standard deviation; n = 18; *$p < 0.001$) were measured. The statistical differences among various concentrations of the same measurement are denoted using different letters.

Table 3. Correlation analysis table for fermented tomato leaves.

	ABTS	DPPH	RP	TPC
ABTS	1	0.64*	0.79*	0.16
DPPH		1	0.90*	0.46
RP			1	0.40
TPC				1

*. Correlation is significant at the 0.01 level (n = 18, two-tailed). Abbreviations: ABTS, ABTS radical scavenging assay; DPPH, DPPH radical scavenging assay; RP, Reducing power assay; TPC, Total phenolic content assay.

Table 4. Plasma biochemical values in rats fed with fermented tomato leaves.

	C (n = 7)	FTL (n = 7)	SEM	p
ALT (U/L)	53.25	57.50	3.31	0.38
AST (U/L)	161.37	177.50	13.77	0.42
Glucose (mg/dL)	219.00	221.13	21.51	0.95
HDL-cholesterol (mg/dL)	23.63	21.00	1.22	0.15
LDL-cholesterol (mg/dL)	12.13	13.13	0.72	0.34
Total bilirubin (mg/dL)	0.05	0.06	0.01	0.55
Creatinine (mg/dL)	0.34	0.34	0.02	0.84
Urea nitrogen (mg/dL)	17.25	18.50	0.64	0.19
Uric acid (mg/dL)	1.23	1.34	0.32	0.80
Total Protein (g/dL)	5.43	5.33	0.21	0.74
Total Cholesterol (mg/dL)	48.88	45.88	1.77	0.25
Triglyceride (mg/dL)	83.88	79.88	10.75	0.80

Statistically significant differences were found, as indicated by the data expressed as least-squares mean (LSMEANS) and standard error of the mean (SEM); n = 7; *p < 0.05. Abbreviations: C, Control group; FTL, Fermented tomato leaves fed group; ALT, Alanine transaminase; AST, Aspartate transaminase; HDL-cholesterol, High-density lipoprotein cholesterol; LDL-cholesterol, Low-density lipoprotein cholesterol.

cholesterol, total bilirubin, creatinine, urea nitrogen, uric acid, total protein, total cholesterol, and triglyceride) in plasma ($p < 0.05$).

Hematological parameters were also analyzed (Table 5) and it was found that FTL did not alter WBC, LYM, MID, GRA, RBC, and PLT parameters. In addition, this diet did not affect HCT, Hb, and MCHC concentrations. Only decreases in MCH and MCV were observed ($p < 0.05$).

No change was observed in TAS and TOS in plasma ($p < 0.05$) (see Table 6).

Compared to the control group, we observed an increasing trend ($p < 0.0001$) in the FI of the test group, which was especially evident on the fifth day and continued until the end of the study. However, there was a difference ($p < 0.0001$) in the upward direction of the water intake (WI) on the first and twelfth days; this difference was not statistically significant on the other days. It was observed that there were differences ($p < 0.05$) in the body weight of the test group compared to the control group during the study period, and the general trend was in the direction of BWG for both groups.

4. Discussion

The total antioxidant capacity can be measured using two main categories of methods: hydrogen atom transfer (HAT)-based and electron transfer (ET)-based methods. The ET-based method measures antioxidant capacity through a color change principle [15]. In the study, the total antioxidant capacity of FTL was measured using four ET-based assays (ABTS, DPPH, RP, and TPC). These methods were chosen as they provide rapid and reproducible results and are comparable to other published reports. The

Table 5. Hematological parameters in rats fed with fermented tomato leaves.

	C (n = 7)	FTL (n = 7)	SEM	p
WBC ($\times10^9$/L)	6.07	5.81	0.77	0.82
LYM (%)	60.17	60.72	2.87	0.89
MID (%)	11.85	12.13	1.29	0.88
GRA (%)	27.99	27.13	2.26	0.79
LYM ($\times10^9$/L)	3.60	3.54	0.51	0.94
MID ($\times10^9$/L)	0.75	1.72	0.84	0.42
GRA ($\times10^9$/L)	1.72	1.55	0.21	0.57
Hb (g/dL)	17.13	17.66	1.10	0.74
MCH (g/dL)	18.74	17.98	0.23	**0.03**
MCHC (g/dL)	36.13	35.77	0.25	0.33
RBC ($\times10^{12}$/L)	9.17	9.88	0.67	0.47
MCV (fL)	51.89	50.21	0.51	**0.03**
RDWs (fL)	13.60	12.87	0.33	0.14
RDWc (fL)	12.69	12.87	0.21	0.55
HCT (%)	47.45	49.41	3.14	0.67
PLT ($\times10^9$/L)	526.38	548.80	50.68	0.76
PCT (%)	0.324	0.333	0.029	0.83
PDWs (%)	7.83	7.82	0.23	0.98
PDWc (%)	31.15	33.98	2.00	0.33
MPV (fL)	6.94	6.09	0.58	0.31

Statistically significant differences were found, as indicated (data expressed as least-squares mean (LSMEANS) and standard error of the mean (SEM); n = 7; *p < 0.05). Abbreviations: C, Control group; FTL, Fermented tomato leaves fed group; WBC, White blood cells; LYM, Lymphocytes; MID, Mid-range absolute count; GRA, Granulocytes; Hb, Hemoglobin; MCH, Mean corpuscular hemoglobin; MCHC, Mean corpuscular hemoglobin concentration; RBC, Red blood cells; MCV, Mean corpuscular volume; RDWs, Red cell distribution width standard deviation; RDWc, Red cell distribution width variation coefficient; HCT, Hematocrit; PLT, Platelets; PCT, Plateletcrit; PDWs, Platelet distribution width standard deviation; PDWc, Platelet distribution width variation coefficient; MPV, Mean platelet volume.

Table 6. Redox parameters in rats fed with fermented tomato leaves.

	C (n = 7)	FTL (n = 7)	SEM	P
TAS (mmol/L)	1.63	1.70	0.08	0.49
TOS (mmol/L)	22.10	16.10	7.13	0.82

The data is presented as least-squares means (LSMEANS) along with their corresponding standard errors of the mean (SEM); n = 7; *p < 0.05. Abbreviations: C, Control group; FTL, Fermented tomato leaves fed group; TAS, Total antioxidant status; TOS, Total oxidant status.

antioxidant capacity of FTL was measured by determining the number of antioxidants using stable synthetic radicals in ABTS and DPPH analyses and by measuring the ferric reducing capacity in the reducing power assay.

In this study, the method of AOA was used to interpret the correlation analysis parameters, taking into consideration the different reaction kinetics of AOA [16].

$ABTS^{\bullet+}$ is a stable radical that is soluble in water and organic solvents, and it is used in the determination of hydrophilic and lipophilic components [11]. Fermented tomato leaf extract showed ABTS radical scavenging in direct proportion to the concentration of up to 5.63 mg/mL. FTL was found to be weaker than Trolox in terms of $ABTS^{\bullet+}$ scavenging (27.0 ± 1.3; 14.8 ± 1.8, respectively).

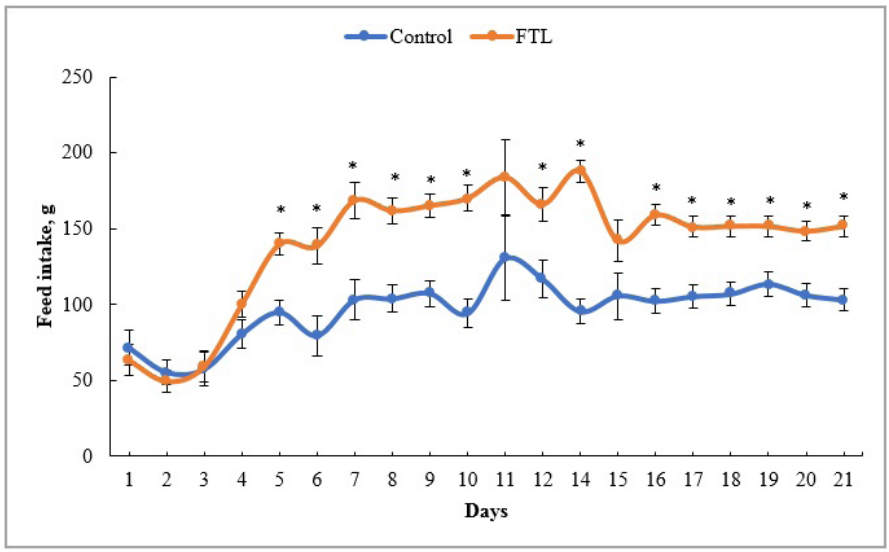

Figure 2. Feed intake responses in rats fed fermented tomato leaves.
The graph shows the change in feed intake of the control and test groups over the study period (data expressed as least-squares mean (LSMEANS) and standard error of the mean (SEM); n = 7; *p < 0.0001).

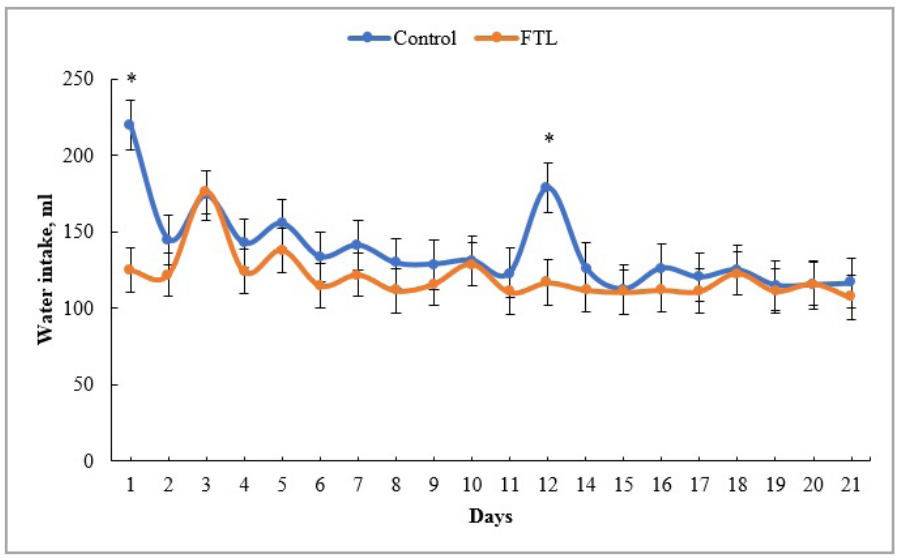

Figure 3. Water intake responses in rats fed fermented tomato leaves.
The graph shows the change in water intake of the control and test groups over the study period (data expressed as least-squares mean (LSMEANS) and standard error of the mean (SEM); n = 7; * p < 0.0001).

DPPH is a relatively stable synthetic organic radical molecule with hydrophilic polarity. Unlike ABTS•+, the DPPH assay is performed in the 515–520 nm range, where carotenes and xanthophylls overlap with DPPH measurements [12]. Despite these disadvantages, the DPPH marker is often used in the determination of AOA [15]. Fermented tomato leaves extract showed DPPH radical scavenging in direct proportion to the concentration up to 11.25 mg/mL, and was found to be much stronger than Trolox in terms of DPPH radical scavenging (5.1 ± 0.8; 68.0 ± 12.7, respectively).

The RP assay is a type of analysis that reflects AOA, and the reduction of Fe^{3+} to Fe^{2+} has been associated with antioxidants acting as electron donors [13]. Fermented

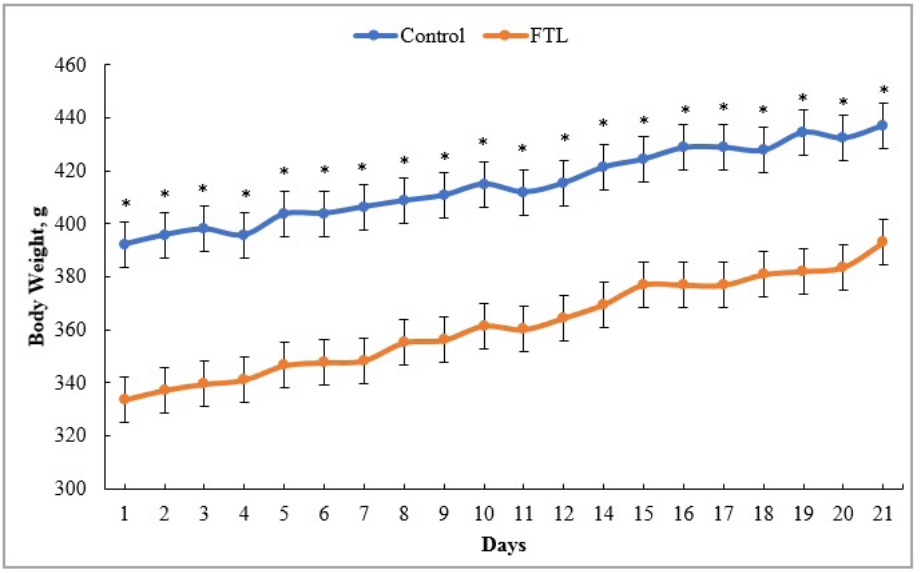

Figure 4. Body weight gain in rats fed fermented tomato leaves.
The graph shows the change in body weight of the control and test groups over the study period (data expressed as least-squares mean (LSMEANS) and standard error of the mean (SEM); n = 7; * $p < 0.05$).

tomato leaves extract performed its reducing power function in direct proportion to the concentration up to 5.63 mg/mL. It was observed that the reducing power capacity of FTL was much stronger than that of Trolox (3.32 ± 0.40; 158.9 ± 0.5, respectively).

A previous study [1] compared the total phenolic and flavonoid contents and antioxidant activities of extracts from different parts of two tomato varieties using ABTS, DPPH, and ORAC assays. The leaf extracts ranked the highest in performance and had a moderately strong positive correlation with TPC in the ABTS, DPPH, and ORAC assays. Our study demonstrates the high antioxidant effects of FTL extracts using ABTS, DPPH, and RP assays, which are similar to those reported in a previous study.

It has been reported that phenolic compounds are responsible for the antioxidant activities of cereals, vegetables, and other botanical materials [17]. To better determine the relationship between AOA and TPC, a TPC assay was performed using the FCR. However, according to the results of correlation analysis, no significant effect of phenolic compounds in FTL on $ABTS^{\bullet+}$ and DPPH radical scavenging nor on RP was detected ($p < 0.01$). The TPC gave weak-moderate positive correlations with ABTS ($R^2 = 0.16$) and DPPH ($R^2 = 0.46$) radical scavenging activity and reducing power ($R^2 = 0.40$). According to a previous study [16], it has been suggested that phenolic compounds in FTL may have weak scavenging activity for ABTS and DPPH radicals and may have weak reductants of ferric ions. In addition, some nonphenolic compounds in FTL may also contribute to total AOA. Other causes of these discrepancies may be different extraction methods and solvents, as well as other slight modifications of the measurements [18]. It has been reported that extraction with polar solvents causes low phenolic [12] and flavonoic yields [19]. Hence, the antioxidant activity of FTL and the potential contribution of its phenolic components may not necessarily correlate with each other in the present study.

It was determined that the DPPH and RP assays and the ABTS assay yielded well-correlated results in terms of radical scavenging activity ($p < 0.01$) (Table 3). This suggested that compounds that scavenge ABTS and DPPH radicals in FTL also have a reduction potential. This is also confirmed by the findings of ABTS and DPPH radical scavenging activity of malting barley varieties [16]. Moreover, our findings are consistent with the report of a previous study [20] that analyzed DPPH and RP levels with tomato pruning axillary green shoots and aerial biomass hydroethanolic extracts. As stated by Pinela [10], the extraction method and the use of water instead of ethanol as a solvent made it possible to determine the high AOA of DPPH and RP.

While the in vitro antioxidant activities of plant-derived components are important for health benefits, the in vivo AOA levels are questionable. This has been attributed to poor bioavailability and metabolic effects on digestive processes [21]. In this study, the expected effect of FTL, which showed high AOA in vitro, was tested in vivo.

In the in vivo phase of this study, rats were fed a diet containing 25% FTL for three weeks. No statistically significant changes were observed in the routine biochemical parameters of the test group compared to the control group. Enzymatic measurements (ALT and AST) and other biochemical parameters were required to evaluate liver function [22]. In our study, liver health status markers such as ALT, AST, and total bilirubin did not show any changes, indicating that the diet we used in rats was not hepatotoxic. However, the hepatoprotective effect of FTL at the dose we used is outside the scope of this study, and more research is needed. It has been reported that tomato leaf protects from atherosclerotic effects thanks to its antioxidant content rich in polyphenols, and in this respect, there is an increase in lipoprotein and cholesterol balance in the direction of HDL-cholesterol and a decrease in LDL-cholesterol and triglyceride [23]. Our study did not observe significant statistical changes in HDL-cholesterol, LDL-cholesterol, total cholesterol, bilirubin, and triglyceride parameters. The results of our study indicate that the lipid profile of the rats was not negatively impacted by the diet they were fed. This could potentially be attributed to the fact that the dosage of FTL used in the study did not have any significant effects on the lipid profile.

Biochemical profiles were used to evaluate acute renal disorders based on creatinine, BUN, uric acid, and total protein [24]. A previous study [25] observed a decrease in urea and creatinine levels, which was attributed to the antioxidant properties of flavonoids in the extract. However, in our study, these parameters did not show any significant difference compared to the control group. Hematology measurements showed that the fermented tomato leaf did not have a hematopathological effect on rats, as previously reported [26], but MCH and MCV values were slightly changed. The extract was found to have immunostimulatory effects, as seen by the increase of WBC, lymphocytes, and platelets in rats [23]. However, more research is needed to fully understand the effects of FTL on hematology.

Redox balance refers to the balance between oxidants and antioxidants. In the study, no significant change in the redox balance was observed in animals fed with the test diet in total oxidant and antioxidant capacity evaluations. Further research is needed on the protective and antioxidant effects of the diet at the used dose.

The rats did not show reluctance in FI and ate the diet with an appetite. One study reported a decrease in FI and BWG from gastrointestinal issues from daily oral administration of different doses of tomato leaf extract [23]. A high concentration of tomatine in the diet causes intestinal issues and has hormetic effects on the organism [27]. The difference observed in the study may be due to the use of tomato leaves in the diet, different dietary components, processing methods, or the hormetic effect depending on the dose used.

The WI profile was generally similar to the control group, indicating that the use of FTL in the test diet did not cause any change in the WI profile. It can be said that the WI was not affected by the feed intake.

The rats allocated to the groups exhibited variations in the average initial weights. To prevent this from affecting the measured parameters, a covariance analysis was performed. Only weight gain during the trial period was considered for interpretation. The results show that the test group had a general increase in body weight.

In conclusion, the fermented tomato leaf diet could not show its in vitro AOA in a monogastric animal model, but adding it to the diet did not harm major vital markers or health profiles. Animals did not refuse to eat the feed and did not experience any adverse effects on BWG. The study provides important findings for using leaf pruning residue in animal diets as a solution to agricultural pollution, but limitations include the need for dose studies and testing its health effects in both monogastric and farm animals.

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The authors declare that they have no conflicts of interest.

Informed consent
Not applicable.

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